Recombinant Human Serpin G1/C1 Inhibitor His-tag

Catalog Number: 10951-PI

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Serpin G1/C1 Inhibitor protein Asn23-Ala500 with a C-terminal 6-His tag Accession # P05155.2
N-terminal Sequence Analysis	Asn23
Predicted Molecular Mass	54 kDa

SPECIFICATIONS

SDS-PAGE	89-98 kDa, under reducing conditions
Activity	Measured by its ability to inhibit Recombinant Human Complement Component C1s (Catalog # 2060-SE) cleavage of a colorimetric peptide substrate, N-carbobenzyloxy-Lys-ThioBenzyl ester (Z-K-SBzl). The IC_{50} is <5 nM, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in Sodium Acetate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij-35 (w/v), pH 7.5 (TCNB)
- Recombinant Human Serpin G1 (Catalog # 10951-PI)
- Recombinant Human Complement Component C1s (rhC1s) (Catalog # 2060-SE)
- Substrate: L-Lys-SBzl (Bachem, Catalog # M-1300), 10 mM stock in DMSO
- 5,5'-Dithobis(2-Nitrozenzoic acid) (DTNB) (Sigma, Catalog # D8130), 10 mM stock in DMSO
- 96-well Clear Plate (Catalog # DY990)
- Fluorescent Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhC1s to 2 µg/mL in Assay Buffer.
2. Prepare a curve of rhSerpin G1 (MW: 54,000 Da) in Assay Buffer. Make the following serial dilutions: 2000, 1000, 500, 100, 50, 25, 12.5, 4, 0.8, and 0.2 nM.
3. Combine 25 µL of diluted rhC1s and 25 µL of rhSerpin G1 at each concentration of the curve. Include a Control containing 25 µL of Assay Buffer and 25 µL of diluted rhC1s.
4. Incubate mixtures at room temperature for 30 minutes.
5. Dilute the mixtures 5-fold by adding 200 µL of Assay Buffer to each reaction and control vial.
6. Dilute the Substrate to 200 µM with Assay Buffer containing 200 µM DTNB.
7. Load into plate 50 µL of the diluted incubated mixtures and start the reaction by adding 50 µL of 200 µM Substrate/200 µM DTNB mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate/ µM DTNB mixture.
8. Read at an absorbance wavelength of 405 nm in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration (IC50) value for rhSerpin G1 by plotting OD/min (or specific activity) versus concentration with 4-PL fitting.
10. The specific activity for rhC1s at each point may be determined using the following formula (if needed):

$$\text{Specific Activity(pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{(ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of the many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhSerpin G1 curve: 100, 50, 25, 5, 2.5, 1.25, 0.625, 0.2, 0.04, and 0.01 nM
- rhC1s: 0.01 µg
- Substrate: 100 µM
- DTNB: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile, deionized water.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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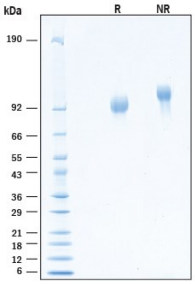
DATA

SDS-PAGE



Recombinant Human Serpin G1 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human Serpin G1 His-tag Protein (Catalog # 10951-PI) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 89-98 kDa.

BACKGROUND

C1-inhibitor (C1-INH), also known as Serpin G1, is a 478 amino acid acute phase plasma glycoprotein member of the serpin superfamily of serine protease inhibitors. C1 inhibitor, like many other serpin family members, contains a highly conserved C-terminal serpin domain with a reactive center loop that protrudes to present a peptide for proteolytic attack (1). Binding disrupts the protease active site and traps it in a covalent complex with C1-inhibitor resulting in inactivation of the protease. C1 inhibitor also contains a highly glycosylated N- terminus and a unique C-terminal tail that has been suggested to serve as a barrier for transition to an inactive latent form (2). C1 inhibitor is synthesized and secreted primarily by hepatocytes but also by other cells such as monocytes, fibroblasts, and macrophages (1). C1 inhibitor is the sole physiological inhibitor of activated classical complement pathway serine proteases C1r and C1s (3). In addition, C1 inhibitor can inhibit multiple other serine proteases including plasma kallikrein and coagulation factor XIIa from the contact activation system, MASP-1 and MASP-2 proteases from the mannose binding lectin pathway, factor XIa and thrombin from the coagulation system, and plasmin and tissue plasminogen activator from the fibrinolytic system (3). Serpin G1 deficiency, whether caused by deletions, mutations, or inactivation, results in an autosomal dominantly inheritable potentially lethal disease, hereditary angioedema (HAE) (1). Some mutations in the SerpinG1 gene are also associated with age-related macular degeneration (4). Given the role C1-inhibitor plays as a potent anti-inflammatory agent, it has been developed for clinical use to treat HAE (1, 5) and considered for treatment of several other conditions including sepsis, acute myocardial infarction, brain injury, transplantation and COVID19 (6-11).

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